Effect of High-Dose Esomeprazole on CYP1A2, CYP2C19, and CYP3A4 Activities in Humans: Evidence for Substantial and Long-lasting Inhibition of CYP2C19

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In vitro, esomeprazole is a time-dependent inhibitor of CYP2C19. Additionally, racemic omeprazole induces CYP1A2 and omeprazole and its metabolites inhibit CYP3A4 *in vitro*. In this 5-phase study, 10 healthy volunteers ingested 20 mg pantoprazole, 0.5 mg midazolam, and 50 mg caffeine as respective index substrates for CYP2C19, 3A4, and 1A2 before and 1, 25, 49 (pantoprazole only), and 73 hours after an 8-day pretreatment with 80 mg esomeprazole twice daily. The area under the plasma concentration-time curve (AUC) of *R*-pantoprazole increased 4.92-fold (90% confidence interval (CI) 3.55–6.82), 2.31-fold (90% CI 1.85–2.88), and 1.33-fold (90% CI 1.06–1.68) at the 1-hour, 25-hour, and 73-hour phases, respectively, consistent with a substantial and persistent inhibition of CYP2C19. The AUC of midazolam increased up to 1.44-fold (90% CI 1.22–1.72) and the paraxanthine/caffeine metabolic ratio up to 1.19-fold (90% CI 1.04–1.36), when the index substrates were taken 1 hour after esomeprazole. Based on the recovery of *R*-pantoprazole oral clearance, the turnover half-life of CYP2C19 was estimated to average 53 hours. Pharmacokinetic simulation based on the observed concentrations of esomeprazole and its metabolites as well as their published CYP2C19 inhibitory constants was well in line with the observed changes in *R*-pantoprazole pharmacokinetics during the course of the study. Extrapolations assuming linear pharmacokinetics of esomeprazole suggested weak to moderate inhibition at 20 and 40 mg twice daily dosing. In conclusion, high-dose esomeprazole can cause strong inhibition of CYP2C19, but only weakly inhibits CYP3A4 and leads to minor induction of CYP1A2. The enzymatic activity of CYP2C19 recovers gradually in ~ 3–4 days after discontinuation of esomeprazole treatment.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Esomeprazole and its metabolites are time-dependent inhibitors of CYP2C19 and some of its metabolites also inhibit CYP3A4. Some studies have suggested that esomeprazole induces CYP1A2.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ This study investigated the effect of high-dose esomeprazole on CYP2C19, CYP1A2, and CYP3A4 activities in humans.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ High-dose esomeprazole causes a substantial, gradually declining inhibition of CYP2C19, which lasts for at least 3 days, consistent with irreversible inhibition. Additionally, it causes a modest CYP3A4-inhibiting and CYP1A2-inducing effect. Using the recovery of *R*-pantoprazole oral clearance, the *in vivo* turnover half-life of CYP2C19 was approximated to be 53 hours.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ Care is warranted if a CYP2C19 substrate drug is used concomitantly or within a few days after discontinuation of esomeprazole. Esomeprazole's effect on CYP3A4 and 1A2 can be clinically relevant for their substrates with narrow therapeutic index. The turnover half-life estimate will be useful in *in vitro-in vivo* extrapolations and physiologically-based pharmacokinetic modeling of CYP2C19 mediated drug-drug interactions.

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Proton pump inhibitors (PPIs) are extensively used to treat stomach acid-related disorders and they are generally well-tolerated.[1] However, their pharmacokinetics vary considerably due to genetic polymorphisms of cytochrome P450 (CYP) 2C19.[2] Moreover, they cause drug-drug interactions (DDIs) by altering drug absorption via increasing gastric pH, and particularly omeprazole and its *S*-enantiomer esomeprazole, by affecting drug metabolism.

Racemic omeprazole and esomeprazole are clinically relevant inhibitors of CYP2C19. In addition, they have been suspected to have an effect on CYP3A4 and CYP1A2 activities. Although *R*-omeprazole inhibits CYP2C19 reversibly, racemic omeprazole and esomeprazole inhibit it metabolism-dependently *in vitro*.[3] In clinical trials, 80 mg omeprazole or 40 mg esomeprazole daily have markedly reduced the CYP2C19-mediated formation of clopidogrel's active metabolite and reduced its antiplatelet effect,[4,5] and modestly raised the plasma concentrations of some other CYP2C19 substrates.[6]

In vitro, omeprazole and its metabolites inhibit CYP3A4.[7,8] In addition, both omeprazole and particularly esomeprazole can activate the pregnane X and aryl hydrocarbon receptors[9,10] and might, therefore, also induce CYP3A4 and CYP1A2 expression. Based on small clinical studies, standard doses of racemic omeprazole slightly increase the concentrations of the CYP3A4 substrates carbamazepine and nifedipine[11,12] suggesting a net inhibitory effect on CYP3A4. Furthermore, clinical trials suggest that racemic omeprazole has a weak CYP1A2 inducing effect in poor metabolizers of CYP2C19 and when used at high doses.[13,14] However, clinical studies of the effects of esomeprazole on CYP3A4 or CYP1A2 activities are sparse and conclusive evidence is lacking. For example, although standard doses of esomeprazole increased the area under the plasma concentration-time curve (AUC) of the CYP3A4 substrate cisapride, it had no effect on the pharmacokinetics of clarithromycin or quinidine, and, in one study, in CYP2C19 poor metabolizers, it had no effect on CYP1A2 activity.[15]

It is important to fully understand the PPIs' effects on CYP2C19, CYP3A4, and CYP1A2, because PPIs are frequently co-administered with many substrates of these CYP enzymes. It is recommended that sensitive index substrates and the highest clinically used doses of the perpetrator drug should be used in clinical DDI studies to characterize the DDI potential of the perpetrator drug.[16] Accordingly, in the case of esomeprazole, the worst-case scenario of its enzyme inhibiting and inducing effects could probably be best estimated by using the high 160 mg daily doses that are occasionally required in Zollinger-Ellison syndrome. For CYP1A2 and CYP3A4, several sensitive index substrates are available, but for CYP2C19, PPIs are the most sensitive. Apart from omeprazole, particularly the *R*-isomer of pantoprazole could be a sensitive index substrate.[17]

As esomeprazole is a metabolism-dependent inhibitor of CYP2C19 *in vitro*,[3] it is likely that high-dose esomeprazole causes a stronger and more persistent inhibition of CYP2C19 than what has been previously observed when using lower esomeprazole doses. Because metabolism-dependent inhibition causes a permanent loss of the enzyme's activity,[18] the recovery of the metabolic function after stopping the treatment with the inhibitor requires *de novo* synthesis of new enzyme. Accordingly, the time needed to reach new steady-state in enzyme activity depends on the enzyme's specific turnover half-life.[19] The turnover of CYP2C19 is poorly characterized, however. The predicted strong metabolism-dependent inhibition of CYP2C19 with high-dose esomeprazole and esomeprazole's relatively short plasma half-life of 1–2 hours provide an excellent opportunity to determine the turnover half-life of CYP2C19 in humans.

The objective of this study was to assess the extent of the inhibitory and inducing effects of esomeprazole on CYP2C19, CYP3A4, and CYP1A2 activities in healthy volunteers after a pretreatment with the highest clinically used daily dose of esomeprazole using pantoprazole, midazolam,[20] and caffeine as respective *in vivo* index substrates (**Figure S1**). The recovery of pantoprazole clearance after stopping esomeprazole dosing was also examined, in order to estimate the turnover half-life of CYP2C19 and to allow mechanistic simulations of the CYP2C19 inhibitory effect of esomeprazole.

METHODS

Study participants

Ten healthy volunteers (5 men and 5 women) were enrolled in the study. All participants gave a written informed consent before any study procedures were performed. Their health was confirmed by medical history, clinical examination, and routine laboratory tests before entering the study. None of the participants were smokers or used any continuous medications (e.g., hormonal contraceptives). The subjects were genotyped for the CYP2C19 alleles **2*, **3*, **8*, and **17*, as decribed in **Supplementary Methods**.

Study design

The study protocol was approved by the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District (record number 57/13/03/00/2015) and by the Finnish Medicines Agency Fimea (EudraCT number 2015-000367-13). A 5-phase fixed-order crossover study was carried out (**Figure S1**). In the control phase (day 1), the participants ingested a 20-mg dose of racemic pantoprazole (Somac 20-mg enteric-coated tablet; Leiras Takeda, Oranienburg, Germany), a 50-mg dose of caffeine (half of a 100-mg tablet of Coffein Etnovia; Etnovia Oy, Seinäjoki, Finland), and 0.5-mg dose of midazolam (0.5 mL of Midazolam Accord 1 mg/mL Solution; Accord Healthcare Limited, Middlesex, UK) with 150 mL of water at 9 AM as probe drugs for CYP2C19, CYP1A2, and CYP3A4 activities, respectively. After finishing the control phase, the participants were given a pretreatment with 80 mg esomeprazole (two Nexium 40-mg enteric-coated tablets; AstraZeneca AB, Södertälje, Sweden) twice daily at 8 AM and 8 PM on days 2–8 with the last dose administered at 8 AM on day 9. Thereafter, the participants were administered 20 mg pantoprazole, 50 mg caffeine, and 0.5 mg midazolam with 150 mL of water 1, 25, and 73 hours after the last esomeprazole dose (days 9, 10, and 12). In addition, 20 mg pantoprazole was administered after 49 hours (day 11), to allow estimation of the AUC of pantoprazole and modeling of the recovery of CYP2C19 activity over the course of the study. After an overnight fast, a standard warm meal and snack were served 3 and 7 hours after the administration of pantoprazole. Alcohol consumption was prohibited for 1 day prior to and during the study days, and caffeine consumption from 9 PM before the days of caffeine administration. The participants were not permitted to consume grapefruits or grapefruit products or use any other medications for 1 week prior to and during the study.

Sampling

Timed blood samples (4 or 9 mL each) were drawn 5 minutes before and 20 minutes, 40 minutes, and 1, 1.5, 2, 3, 4, 5, and 7 hours after

pantoprazole administration in the control phase and on the days when pantoprazole was administered 1, 25, or 73 hours after the last esomeprazole dose. A blood sample was also drawn just before the administration of the last esomeprazole dose. On the day pantoprazole was administered 49 hours after the last esomeprazole dose, blood samples were drawn only 5 minutes before, and 2 and 7 hours after pantoprazole ingestion. The samples were drawn in tubes containing ethylenediaminetetraacetic acid and placed on ice immediately after sampling. Plasma was separated within 30 minutes and stored in −70°C until analysis.

Pharmacokinetics

Methods for determination of drug concentrations are described in **Supplementary Methods** and **Table S1**. The following pharmacokinetic variables were calculated for pantoprazole, midazolam, and their metabolites by standard noncompartmental methods using Phoenix WinNonlin, version 6.4 (Certara, Princeton, NJ): peak plasma concentration (C_{max}), time to C_{max} (T_{max}), AUC from 0–7 hours ($AUC_{0-7\ h}$), AUC from 0 hours to infinity ($AUC_{0-\infty}$), and terminal half-life ($t_{1/2}$). Furthermore, the C_{max}, $t_{1/2}$, and fractional AUCs corresponding to each phase after the last dose of esomeprazole ($AUC_{0-8\ h}$, $AUC_{25-32\ h}$, $AUC_{49-56\ h}$, and $AUC_{73-80\ h}$) were calculated for esomeprazole and its metabolites. The oral clearances of the enantiomers of pantoprazole were calculated by first dividing the oral dose of racemic pantoprazole (20 mg) by two and then by their respective $AUC_{0-\infty}$ values. To assess CYP1A2 activity, the paraxanthine/caffeine ratio was calculated from a blood sample taken 5 hours after caffeine dosing.[21]

Statistical analysis

Ten subjects were estimated to be adequate to detect a 30% change in $AUC_{0-\infty}$ between the control and the following phases with a power of at least 80% (α level 5%). The results are expressed as geometric means and geometric mean ratios with geometric coefficient of variations or 90% confidence intervals (CIs), unless otherwise stated. All pharmacokinetic variables, except T_{max}, were logarithmically transformed before statistical analysis. The pharmacokinetic variables were compared by repeated-measures analysis of variance with the study phase as a within-subjects factor, with pairwise comparisons with the Fisher's least significant difference method. P values < 0.05 were considered statistically significant. Wilcoxon signed rank test was used for comparisons of T_{max}. Statistical analyses were carried out using IBM SPSS Statistics for Mac OS (version 24.0.0.0; IBM, Armonk, NY).

Estimation of CYP2C19 enzyme turnover half-life

The CYP2C19 enzyme turnover half-life was estimated by regression analysis, using the recovery of the oral clearances of *R*-pantoprazole after esomeprazole administration, as described previously (**Supplementary Methods**).[22]

Simulation of CYP2C19 activity following esomeprazole dosing

The time course of CYP2C19 inhibition during the study was modeled using dynamic methods and numerical solutions, as previously described (**Supplementary Methods** and **Table S2**) and carried out in MS Excel for Mac (version 16.36, Microsoft, Redmont, WA).[23] For the simulations, the degradation half-life of CYP2C19 was set as 53 hours (0.0131 hr^{-1}) and the liver CYP2C19 expression level in the baseline condition was assumed to be 14 pmol/mg microsomes.[24] The previously published[7] reversible and time-dependent inhibition kinetic values for racemic omeprazole and its metabolites were used to predict the time course and magnitude of CYP2C19 inhibition. The K_I-values for each compound were corrected for the competition of binding by other circulating compounds, as previously described,[25] and the overall effect on CYP2C19 activity was predicted simultaneously accounting for esomeprazole and its metabolites assuming the inactivation followed an additive model.[25] For modeling purposes, the observed plasma concentrations of esomeprazole and its metabolites during the dose interval on the last day of the pretreatment were used throughout the time course of the simulation for each administered dose (interval). For each time point, the unbound concentrations of each compound were calculated based on previously reported plasma unbound fractions.[7] The observed minor accumulation of the metabolites, particularly the sulfone, was not considered in the quantitative predictions. In addition, assuming linear pharmacokinetics of esomeprazole, extrapolations for 40 mg or 20 mg twice daily dosing regimens were made by multiplying the observed plasma concentrations of each compound with 50% and 25%, respectively.

RESULTS

Pharmacokinetics of pantoprazole

Esomeprazole increased *R*-pantoprazole $AUC_{0-\infty}$ 4.92-fold ($P < 0.001$), 2.31-fold ($P < 0.001$), and 1.33-fold ($P < 0.05$) compared with the control when pantoprazole was administered 1, 25, and 73 hours after the last esomeprazole dose, respectively (**Figure 1b**, **Figure 2a**, **Table 1**). *S*-pantoprazole $AUC_{0-\infty}$ was increased 2.05-fold (P < 0.005) and 1.63-fold ($P < 0.05$) in the 1 and 25 hour phases, respectively (**Figure 1a**, **Table 1**), whereas the $AUC_{0-\infty}$ was not significantly increased in the 73 hour phase. In the 1, 25, and 73 hour phases, the C_{max} of *R*-pantoprazole was increased 2.68-fold ($P < 0.001$), 1.97-fold ($P < 0.05$), and 1.53-fold ($P < 0.05$), and $t_{1/2}$ was prolonged from 0.8 to 2.7 hours ($P < 0.001$), 1.4 hours (90% CI, 1.5–2.0-fold; $P < 0.001$), and 1.0 hours ($P < 0.001$), respectively. The C_{max} of *S*-pantoprazole was increased 1.90-fold ($P < 0.05$) and 1.60-fold ($P < 0.05$) in the 1 and 25 hour phases. The $t_{1/2}$ of *S*-pantoprazole was prolonged from 1.0 to 1.6 hours ($P < 0.001$), 1.4 hours ($P < 0.001$), and 1.2 hours ($P < 0.005$) in the 1, 25, and 73-hour phases, respectively.

Pharmacokinetics of midazolam

Esomeprazole increased midazolam $AUC_{0-\infty}$ 1.44-fold ($P < 0.005$) and C_{max} 1.59-fold ($P < 0.005$), when midazolam was administered 1 hour after esomeprazole (**Figure 1c**, **Figure 2b**, **Table 1**). The corresponding 1′-hydroxymidazolam/midazolam $AUC_{0-\infty}$-ratio was decreased to 77% of control ($P < 0.001$; **Figure 2c**). Esomeprazole had no significant effect on the $t_{1/2}$ of midazolam. No significant changes in the pharmacokinetics of midazolam or 1′-hydroxymidazolam were observed compared with control, when midazolam was administered 25 or 73 hours after esomeprazole, except for a 35% increase in the C_{max} of midazolam in the 73-hour phase ($P < 0.05$).

Paraxanthine/caffeine ratio

The paraxanthine/caffeine concentration ratio was increased 1.19-fold ($P < 0.05$; **Figure 2d**, **Table 1**) when caffeine was ingested 1 hour after the last dose of esomeprazole, but the ratio was not significantly increased at later time points.

Pharmacokinetics of esomeprazole

The C_{max} of esomeprazole and its metabolites after the last esomeprazole dose occurred at 1.7–3.0 hours (**Figure 3**, **Table 2**).

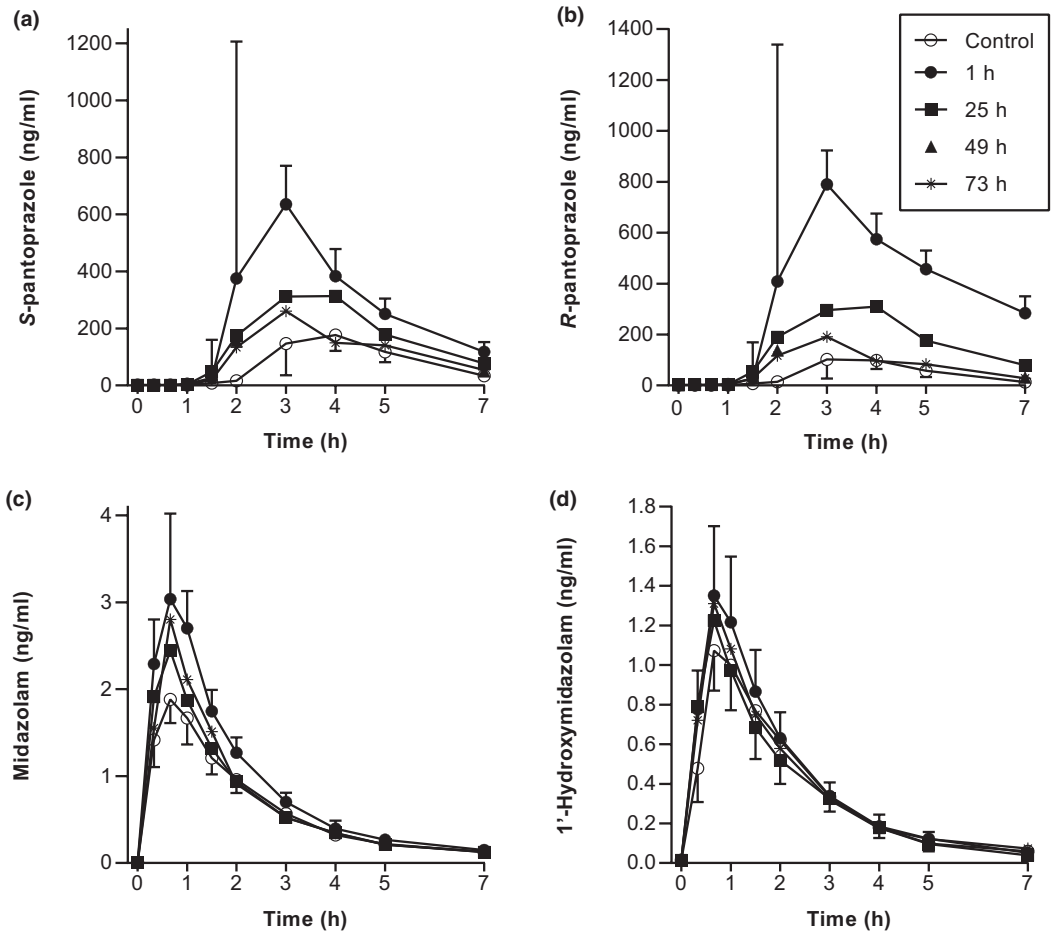

Figure 1 The plasma concentrations of pantoprazole enantiomers, midazolam and 1′-hydroxymidazolam. The panels show the geometric mean plasma concentrations with 90% confidence intervals of (**a**) *S*-pantoprazole and (**b**) *R*-pantoprazole, (**c**) midazolam and its metabolite (**d**) 1′-hydroxymidazolam following a single-dose of 20 mg of racemic pantoprazole and 0.5 mg of midazolam at 9 AM in 10 healthy volunteers. Pantoprazole was administered before (control) and 1, 25, 49, and 73 hours after, and midazolam before and 1, 25, and 73 hours after the last esomeprazole dose of an 8-day pretreatment with 80 mg esomeprazole twice daily. For clarity, some error bars have been omitted.

At 25 hours after the last esomeprazole dose, the plasma concentrations of esomeprazole had decreased below the detection limit (10 ng/mL; i.e., to < 1% of the average C_{max} in each subject). At 25 hours, the mean concentrations of 5'-*O*-desmethylomeprazole sulfide and omeprazole sulfone were 4.6% and 3.1% of their peak, respectively, and the other metabolite concentrations were ≤ 1% of their peak (**Figure 3**).

CYP2C19 genotypes

There was a trend toward a larger increase in the exposure to *R*-pantoprazole in subjects with higher activity *CYP2C19*-genotypes (**1/*17* and **1/*1*) than in subjects with the **1/*2* genotype, with a 16.6-fold increase in the $AUC_{0-\infty}$ of *R*-pantoprazole in one subject with the *CYP2C19*1/*17* genotype (**Figure 4**, **Table S3**). However, no association was evident between *CYP2C19*-genotypes and AUC values of esomeprazole or *S*-pantoprazole. No formal statistical comparisons were performed between the genotype groups due to the small sample size.

CYP2C19 turnover half-life and simulation of the time course of CYP2C19 activity

The estimated CYP2C19 turnover half-life was 53.3 hours based on pooled data of all subjects after exclusion of one subject whose individual data had poor statistical fit. When the subjects were analyzed separately, the geometric mean turnover half-life was 49.5 hours (**Figure 5**, **Table S3**). When the time course of CYP2C19 activity following esomeprazole dosing was simulated based on the concentrations of esomeprazole and its metabolites and published *in vitro* CYP2C19 inhibitory activity of racemic omeprazole and its metabolites,[7] the simulated magnitude of CYP2C19 inhibition and the time course of recovery of CYP2C19 activity were in good agreement with the observed data of *R*-pantoprazole clearance (**Figure 5**).

DISCUSSION

This study showed that high-dose esomeprazole treatment leads to substantial, from moderate to strong inhibition of CYP2C19, resulting in an average fivefold increase in the AUC

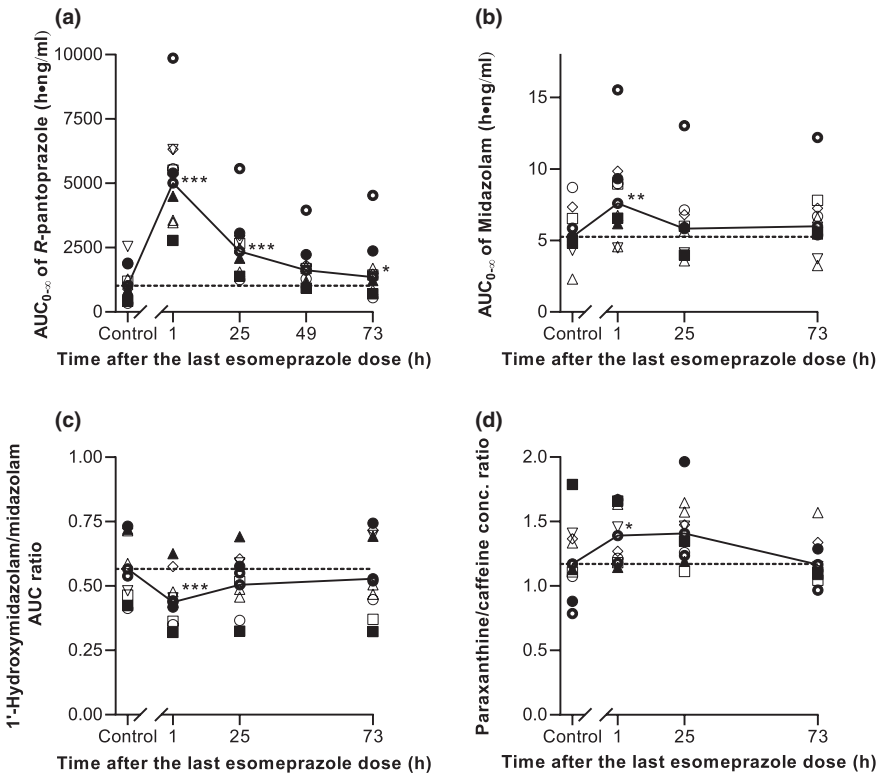

Figure 2 The area under the plasma concentration-time curve from zero to infinity ($AUC_{0-\infty}$) of R-pantoprazole and midazolam, the $AUC_{0-\infty}$ ratio of 1'-hydroxymidazolam/midazolam and the paraxanthine/caffeine concentration ratio over the course of the study. The panels show the geometric mean and individual body weight adjusted (to 70 kg) $AUC_{0-\infty}$ values of (**a**) *R*-pantoprazole and (**b**) midazolam, (**c**) the 1′-hydroxymidazolam/midazolam $AUC_{0-\infty}$ ratio, and (**d**) the 5-hour postdose paraxanthine/caffeine concentration ratio before and after the last esomeprazole dose. A 20-mg dose of pantoprazole was administered before (control) and 1, 25, 49, and 73 hours after, and a 0.5-mg dose of midazolam and a 50-mg dose of caffeine before and 1, 25, and 73 hours after an 8-day pretreatment with 80 mg esomeprazole twice daily. Individual data are denoted with different symbols, and geometric means are connected with a solid line between study phases. * $P < 0.05$ vs. control; ** $P < 0.005$ vs. control; *** $P < 0.001$ vs. control.

of the *R*-enantiomer of pantoprazole. Of note, the increases in *R*-pantoprazole AUC were more than fivefold in most individuals who were rapid or normal CYP2C19 metabolizers (noncarriers of the no-function *CYP2C19* alleles). Albeit smaller, the effect of esomeprazole on pantoprazole persisted until 73 hours after esomeprazole dosing. In addition, esomeprazole slightly raised the plasma concentrations of the CYP3A4-substrate midazolam and the paraxanthine/caffeine ratio, an index of CYP1A2 activity, when midazolam and caffeine were administered 1 hour after esomeprazole.

According to *in vitro-in vivo* extrapolations, the inhibitory effect of racemic omeprazole on CYP2C19 is to a large extent explained by time-dependent inhibition by parent omeprazole and its metabolites omeprazole sulfone and 5′-*O*-desmethylomeprazole,[3,7] which have been estimated to contribute by about 70%, 5%, and 25% to the total CYP2C19 inhibition, respectively.[7] The simulations and the concentrations measured in this high-dose study predicted that esomeprazole contributes only 50% to the total CYP2C19 inactivation, whereas the sulfone and desmethylomeprazole contribute about 20–30% each (data not shown). This is likely due to the nonlinear kinetics of esomeprazole and subsequent different metabolite-to-parent ratios at different doses; due to autoinhibition of the CYP2C19-mediated metabolism of omeprazole, the proportion of the CYP3A4-dependent omeprazole sulfone increases dose-dependently and time-dependently.[26] Additionally, according to *in vitro* and clinical studies, the sulfone metabolite is quantitatively more important in the metabolism of esomeprazole than in that of racemic omeprazole.[27,28] In turn, metabolites of omeprazole have been estimated to account for > 60% of the inhibition of CYP3A4, mainly due to metabolism-dependent inactivation of CYP3A4 by 5′-*O*-desmethylomeprazole and possibly reversible CYP3A4 inhibition by 5′-hydroxyomeprazole.[7]

In the present study, the inhibitory effect of esomeprazole on CYP2C19 was relatively strong at 25 hours after esomeprazole dosing, even though the concentrations of the main CYP2C19 inhibiting compounds esomeprazole and 5′-*O*-desmethylomeprazole had decreased to < 1% of their peak and those of omeprazole sulfone to about 3% of its peak. As also observed in the simulation, it is likely that the inactivation process of CYP2C19 had ceased practically completely and that the direct inhibitory effects of esomeprazole and its metabolites were not clinically relevant at that time. Accordingly, the present results demonstrate that esomeprazole can cause a long-lasting inhibitory effect on CYP2C19, consistent with an irreversible or mechanism-based inhibitory effect on CYP2C19. As the recovery of enzyme activity after

Table 1 Pharmacokinetic variables of pantoprazole, midazolam and 1′-OH-midazolam following a single 20-mg oral dose of pantoprazole and a single 0.5-mg oral dose midazolam in 10 healthy subjects after the last dose of an 8-day pretreatment with a 80-mg dose of esomeprazole twice daily, when pantoprazole was administered 1, 25, or 73 hours after the last esomeprazole dose

		Time from the last esomeprazole dose		
Variable	Control	1 hour	25 hours	73 hours
S-pantoprazole				
C_{max}, ng/mL	495 (69)	943 (28)*	789 (27)*	689 (42)†
Ratio to control, 90% CI	–	1.90 (1.29–2.80)	1.60 (1.11–2.30)	1.39 (1.04–1.86)
$t_{1/2}$, hours	1.0 (18)	1.6 (15)***	1.4 (18)***,†	1.2 (13)**,†††,††
Ratio to control, 90% CI	–	1.60 (1.42–1.82)	1.36 (1.22–1.52)	1.22 (1.11–1.34)
$AUC_{0\text{-}7\ h}$, ng·h/mL	1275 (67)	2489 (26)**	2047 (31)*,†	1605 (46)††,††
Ratio to control, 90% CI	–	1.95 (1.40–2.73)	1.60 (1.23–2.09)	1.26 (0.99–1.60)
$AUC_{0\text{-}\infty}$, ng·h/mL	1351 (65)	2771 (27)**	2205 (33)*,††	1708 (47)†††,†††
Ratio to control, 90% CI	–	2.05 (1.48–2.85)	1.63 (1.26–2.11)	1.26 (1.00–1.60)
R-pantoprazole				
C_{max}, ng/mL	387 (66)	1037 (26)***	761 (31)*,†	592 (45)*,†††,††
Ratio to control, 90% CI	–	2.68 (1.87–3.84)	1.97 (1.40–2.78)	1.53 (1.12–2.08)
$t_{1/2}$, hours	0.8 (20)	2.7 (21)***	1.4 (26)***,†††	1.0 (16)***,†††,***,†††
Ratio to control, 90% CI	–	3.25 (2.80–3.76)	1.76 (1.54–1.99)	1.26 (1.15–1.38)
$AUC_{0\text{-}7\ h}$, ng·h/mL	886 (66)	3390 (24)***	1916 (32)***,†††	1171 (56)*,†††,†††
Ratio to control, 90% CI	–	3.83 (2.77–5.28)	2.16 (1.73–2.71)	1.32 (1.05–1.66)
$AUC_{0\text{-}\infty}$, ng·h/mL	916 (65)	4504 (28)***	2113 (38)***,†††	1219 (57)*,†††,†††
Ratio to control, 90% CI	–	4.92 (3.55–6.82)	2.31 (1.85–2.88)	1.33 (1.06–1.68)
Midazolam				
C_{max}, ng/mL	2.07 (29)	3.31 (38)**	2.50 (27)†	2.81 (37)*,†
Ratio to control, 90% CI	–	1.59 (1.31–1.96)	1.21 (1.02–1.43)	1.35 (1.08–1.69)
$t_{1/2}$, hours	1.5 (9.2)	1.4 (14)	1.5 (4.8)	1.5 (13)
Ratio to control, 90% CI	–	0.90 (0.82–0.99)	0.96 (0.91–1.01)	0.94 (0.86–1.03)
$AUC_{0\text{-}7\ h}$, ng·h/mL	4.49 (26)	6.38 (28)***	4.93 (26)†††	5.18 (31)††
Ratio to control, 90% CI	–	1.42 (1.23–1.64)	1.10 (0.94–1.28)	1.15 (0.99–1.34)
$AUC_{0\text{-}\infty}$, ng·h/mL	4.72 (27)	6.82 (31)**	5.23 (30)†††	5.39 (32)†††
Ratio to control, 90% CI	–	1.44 (1.22–1.72)	1.11 (0.93–1.32)	1.14 (0.97–1.34)
1′-OH-Midazolam				
C_{max}, ng/mL	1.21 (34)	1.39 (42)	1.23 (47)	1.35 (39)
Ratio to control, 90% CI	–	1.15 (0.94–1.41)	1.02 (0.83–1.25)	1.12 (0.93–1.34)
$t_{1/2}$, hours	1.4 (20)	1.3 (16)	1.4 (21)	1.5 (12)
Ratio to control, 90% CI	–	0.96 (0.88–1.04)	0.98 (0.88–1.08)	1.05 (0.97–1.14)
$AUC_{0\text{-}7\ h}$, ng·h/mL	2.57 (29)	2.89 (36)	2.54 (41)	2.72 (33)
Ratio to control, 90% CI	–	1.13 (0.98–1.30)	0.99 (0.81–1.21)	1.06 (0.92–1.22)
$AUC_{0\text{-}\infty}$, ng·h/mL	2.67 (29)	2.98 (36)	2.64 (42)	2.84 (33)
Ratio to control, 90% CI	–	1.12 (0.97–1.29)	0.99 (0.80–1.21)	1.06 (0.92–1.23)
1′-OH-M/M $AUC_{0\text{-}7\ h}$ ratio	0.57 (24)	0.45 (22)***	0.52 (25)†	0.53 (30)†
Ratio to control, 90% CI	–	0.79 (0.72–0.87)	0.90 (0.80–1.02)	0.92 (0.82–1.04)
1′-OH-M/M $AUC_{0\text{-}\infty}$ ratio	0.57 (24)	0.44 (21)***	0.50 (24)†	0.53 (30)†
Ratio to control, 90% CI	–	0.77 (0.71–0.85)	0.89 (0.80–0.99)	0.93 (0.83–1.05)

(Continued)

Table 1 (Continued)

Variable	Control	Time from the last esomeprazole dose		
		1 hour	25 hours	73 hours
Caffeine				
Paraxanthine/caffeine concentration ratio	1.17 (24)	1.39 (16)*	1.41 (17)	1.17 (14)
Ratio to control, 90% CI	–	1.19 (1.04–1.36)	1.20 (1.01–1.42)	1.00 (0.87–1.14)

Data are given as geometric mean with geometric coefficient of variation. The geometric mean ratios between the two phases are given with 90% CI.
$AUC_{0\text{-}7\ h}$, area under the plasma concentration-time curve from time 0 to 7 hours; $AUC_{0\text{-}\infty}$, area under the plasma concentration-time curve from time 0 to infinity; CI, confidence interval; C_{max}, peak plasma concentration; $t_{1/2}$, elimination half-life.
*$P < 0.05$ vs. control.
**$P < 0.005$ vs. control.
***$P < 0.001$ vs. control.
$^{\dagger}P < 0.05$ vs. 1 hour.
$^{\dagger\dagger}P < 0.005$ vs. 1 hour.
$^{\dagger\dagger\dagger}P < 0.001$ vs. 1 hour.
$^{\ddagger}P < 0.05$ vs. 25 hours.
$^{\ddagger\ddagger}P < 0.005$ vs. 25 hours.
$^{\ddagger\ddagger\ddagger}P < 0.001$ vs. 25 hours.

mechanism-based inhibition occurs by *de novo* synthesis of the enzyme, likely occurring at a constant rate, it was possible to calculate the *in vivo* turnover half-life of CYP2C19, giving an estimate of ~ 53 hours.

Mainly based on *in vitro* data and indirect *in vivo* models, the turnover half-lives of CYP enzymes have generally been estimated to range from 23–140 hours,[18] showing large differences between enzymes and methods used. For example, the *in vivo* estimates of CYP3A4 turnover half-life have ranged from 70–140 hours,[19] whereas that of CYP2C8 has been only 22 hours.[22] However, *in vivo* data concerning the turnover of some other CYPs, such as CYP2C19, have been sparse. A small study with human hepatic slices from three nonrelated livers suggested that the half-life of CYP2C19 is between 7 and 50 hours.[29] Our estimated CYP2C19

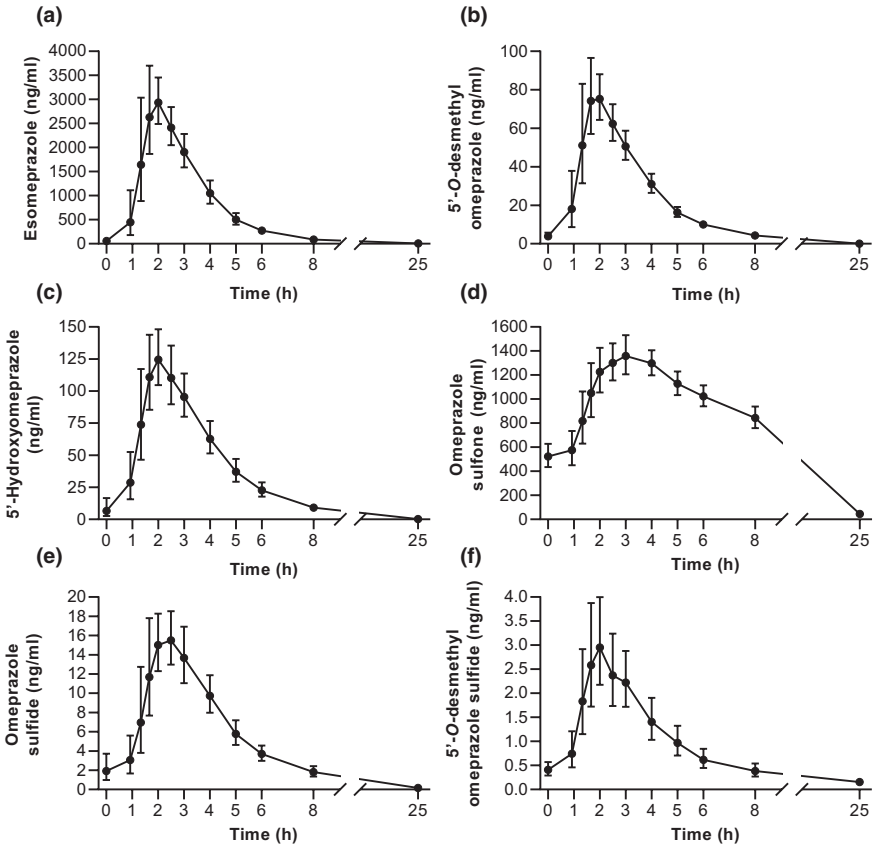

Figure 3 The geometric mean plasma concentrations with 90% confidence intervals of esomeprazole and its metabolites after the last dose of an 8-day pretreatment with 80 mg of esomeprazole twice daily. (**a**) Esomeprazole, (**b**) 5′-*O*-desmethyl-omeprazole, (**c**) 5′-OH-omeprazole, (**d**) omeprazole sulfone, (**e**) omeprazole sulfide, and (**f**) 5′-*O*-desmethyl-omeprazole sulfide.

Table 2 Pharmacokinetic variables of esomeprazole (*S*-OME) and its metabolites 5'-*O*-desmethyl-omeprazole (5-*O*-dmet-OME), 5'-OH-omeprazole (5-OH-OME), omeprazole sulfone (OME-SO_2), omeprazole sulfide (OME-S), and 5'-*O*-desmethyl-omeprazole sulfide (5-*O*-dmet-OME-S) after the last dose of an 8-day pretreatment with 80 mg of esomeprazole twice daily

	C_{max}, ng/mL	T_{max}, hour	$t_{1/2}$, hour	$AUC_{0\text{-}8\ h}$, ng·h/mL	$AUC_{0\text{-}\infty}$, ng·h/mL	$AUC_{25\text{-}32\ h}$, ng·h/mL	$AUC_{49\text{-}56\ h}$, ng·h/mL	$AUC_{73\text{-}80\ h}$, ng·h/mL
S-OME	3,360 (30)	1.66 (1.33–2.5)	1.2 (14)	8,410 (28)	8,570 (28)	0.00	0.00	0.00
5-*O*-dmet-OME	86.0 (29)	1.66 (1.33–2.0)	1.4 (13)	241 (21)	250 (20)	0.00	0.00	0.00
5-OH-OME	135 (30)	1.66 (1.33–3.0)	1.7 (20)	431 (30)	456 (31)	0.02 (660)	0.00	0.00
OME-SO_2	1,435 (18)	3.00 (2.0–4.0)	4.1 (18)	8,353 (16)	13,380 (23)	158.6 (100)	2.27 (250)	0.04 (1200)
OME-S	18.3 (36)	2.50 (1.33–3.0)	2.9 (39)	59.6 (37)	70.3 (36)	0.15 (2,900)	0.00	0.00
5-*O*-dmet-OME-S	3.37 (60)	1.83 (1.33–2.5)	7.8 (40)	10.5 (50)	17.1 (44)	0.74 (44)	0.09 (200)	0.01 (970)

Data are given as geometric mean with geometric coefficient of variation (CV%) except for T_{max}, which is given as median with range.
$AUC_{0\text{-}\infty}$, area under the plasma concentration-time curve from zero to infinity; $AUC_{0\text{-}8\ h}$, area under the plasma concentration-time curve from zero to 8 hours; $AUC_{25\text{-}32\ h}$, area under the plasma concentration-time curve from 25 to 32 hours; $AUC_{46\text{-}56\ h}$, area under the plasma concentration-time curve from 46 to 56 hours; $AUC_{73\text{-}80\ h}$, area under the plasma concentration-time curve from 73 to 80 hours; C_{max}, peak plasma concentration; T_{max}, time to peak plasma concentration; $t_{1/2}$, terminal half-life.

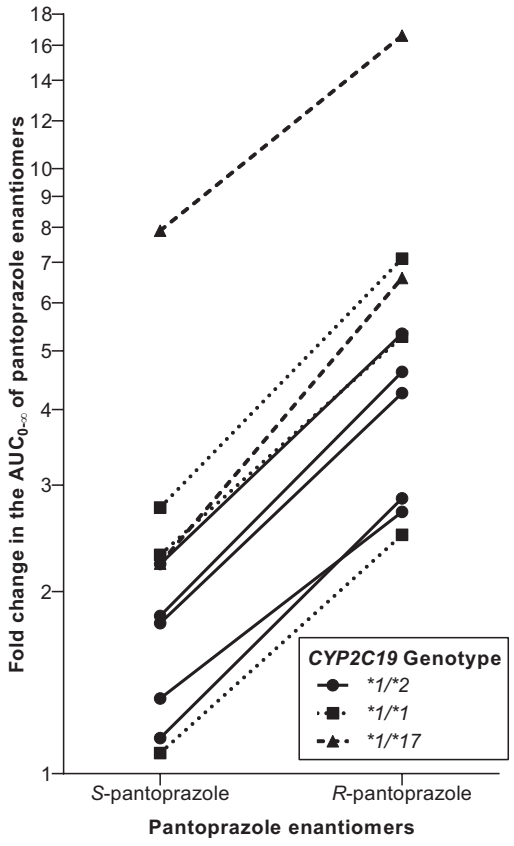

Figure 4 The individual fold increases in the area under the plasma concentration-time curve from zero to infinity ($AUC_{0\text{-}\infty}$) of S-pantoprazole and *R*-pantoprazole following administration of 20 mg of racemic pantoprazole 1 hour after the last esomeprazole dose. Pantoprazole was administered 1 hour after the last dose of an 8-day pretreatment with 80 mg of esomeprazole twice daily. In the control phase, pantoprazole was administered without pretreatment. CYP2C19 genotypes are indicated with the following symbols: circle and solid lines for **1/*2*, squares for **1/*1*, and triangles for **1/*17*. *R*-pantoprazole was more sensitive to alterations in CYP2C19 activity and its ratios ranged from 2.5-fold to 16.6-fold depending on the genotype.

half-life of 53 hours is longer than these *in vitro* values and likely to be more useful in modeling of CYP2C19 inhibition and induction. Moreover, this result suggests that CYP2C19 activity recovers slower than that of the structurally related CYP2C8[22] and comparably with, for example, CYP1A2, CYP2D6, and CYP2E1.[19]

Our findings implicate markedly stronger inhibition of CYP2C19 by esomeprazole than what has been observed previously with smaller esomeprazole doses. In the previous studies, the greatest alteration in the AUC value of a CYP2C19 substrate caused by esomeprazole (30 mg daily) has been a 1.8-fold increase in diazepam exposure.[15] For comparison, 40 mg racemic omeprazole has increased the AUC of diazepam 2.2-fold,[30] and that of moclobemide 2.2-fold in normal CYP2C19 metabolizers.[31] The stronger effect of esomeprazole seen in the present study is mainly explained by the 4–5 times higher 160 mg daily dose used. In addition, pantoprazole is likely a more sensitive CYP2C19 index substrate than diazepam or moclobemide, allowing even 5-fold to 10-fold increases in the AUC to occur.[16]

Based on pharmacogenetic studies comparing normal and poor metabolizers of CYP2C19, it can be estimated that the average contribution of CYP2C19 to *R*-pantoprazole clearance is > 85% in normal metabolizers.[17,32] Our dynamic model suggested that, during the 80 mg twice daily dosing, esomeprazole causes about 75% inhibition of CYP2C19, and full recovery of CYP2C19 activity can be obtained within 1 week after stopping the treatment. An extrapolated simulation with the more commonly used doses of 40 mg and 20 mg twice daily predicted about 60% and 40% inhibition of CYP2C19 at steady-state, respectively. However, because we had to assume linear pharmacokinetics of esomeprazole, as no sufficient pharmacokinetic data of esomeprazole and its metabolites at lower doses were available, these extrapolations should be interpreted with caution. Although the simulated time-course of CYP2C19 activity after 80 mg twice daily dosing was generally in very good agreement with the observed changes in *R*-pantoprazole clearance, particularly during recovery of CYP2C19 activity, the simulations cannot fully explain the changes in *R*-pantoprazole pharmacokinetics, even if

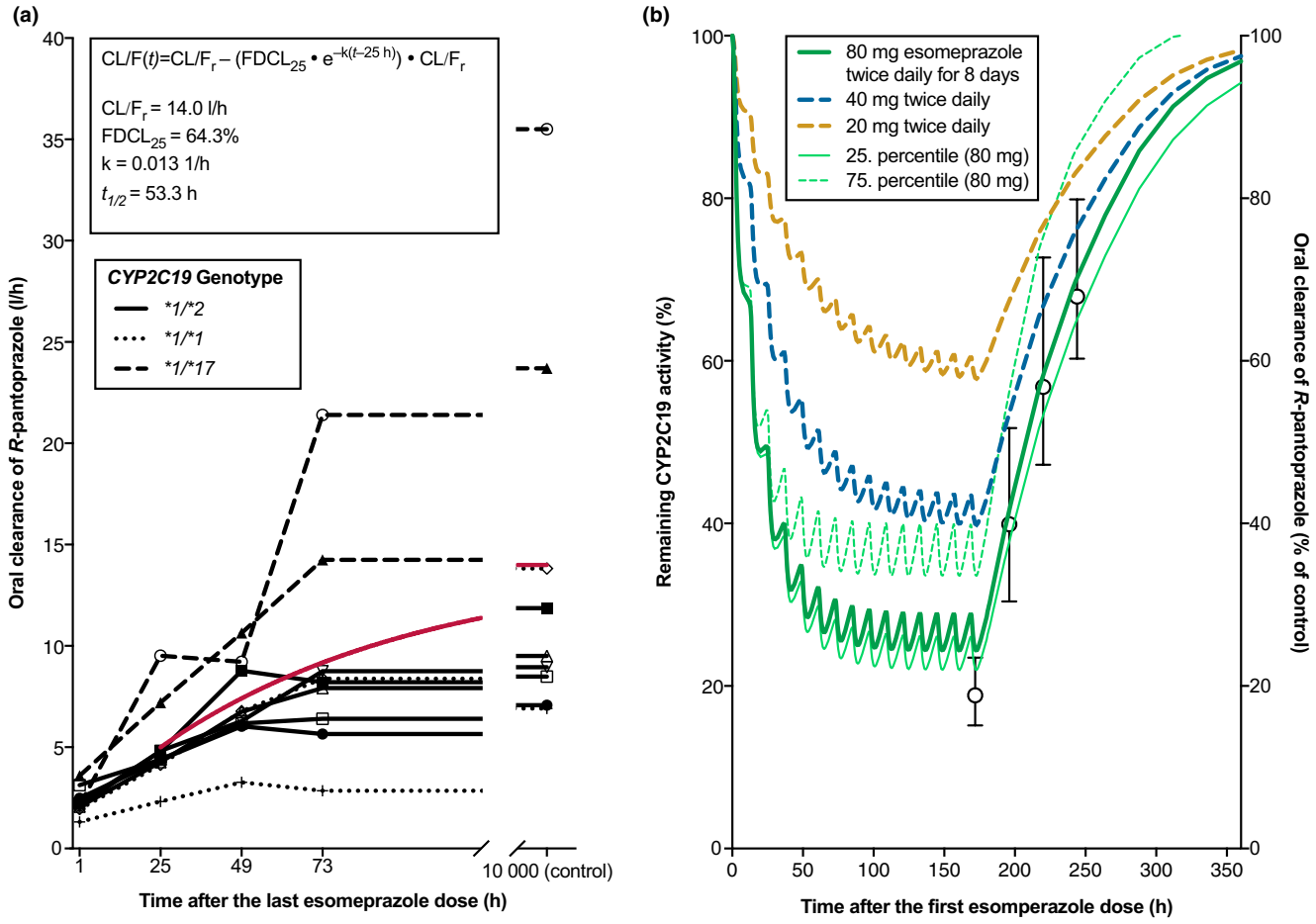

Figure 5 Estimation of the turnover half-life of CYP2C19 and simulation of CYP2C19 activity during and after an 8-day treatment with esomeprazole. (**a**) Estimation of the turnover half-life of CYP2C19 using a nonlinear regression model based on the recovery of the oral clearance (CL/F) of *R*-pantoprazole. A 20-mg dose of racemic pantoprazole was administered before and 1, 25, 49, and 73 hours after the last 80-mg dose of esomeprazole twice daily to 10 healthy volunteers. The red line represents the estimate based on the pooled data of the subjects. For detailed description of the regression model, see the **Supplementary Table S2**. Individual CL/Fs are visualized and *CYP2C19* genotypes are denoted with different line types. (**b**) Simulated time course of CYP2C19 activity after time-dependent and reversible inhibition caused by esomeprazole and its metabolites over the course of the study. The solid green line represents the remaining CYP2C19 activity after 80 mg esomeprazole twice daily for 8 days based on dynamic modeling using the estimated average degradation rate constant (k_{deg}) of CYP2C19 as described in the Methods section. The thin green lines represent the 25th and the 75th percentiles of individual CYP2C19 degradation rate constants. The hollow circles and error bars represent the geometric mean, 25th, and 75th percentiles of individual CL/F values of *R*-pantoprazole in the 1, 25, 49, and 73 hours phases as a percentage of the CL/F in the control phase. CL/F values are plotted at 3 hours after the administration of the 20-mg pantoprazole dose in each phase. Dashed blue and yellow lines represent extrapolations of CYP2C19 activity after 40 mg or 20 mg twice daily dosing regimens, assuming linear pharmacokinetics of esomeprazole. $t_{1/2}$, terminal half-life.

the contribution of CYP2C19 to its clearance was almost 100%. In the present simulations, we used racemic omeprazole's inhibition constants. Thus, a possible explanation for this is that esomeprazole is a stronger time-dependent inhibitor of CYP2C19 than *R*-omeprazole, as suggested by previous *in vitro* findings.[3] Moreover, the unbound concentrations of esomeprazole in hepatocytes could be higher during its absorption phase than those in peripheral plasma. In addition, it is possible that the CYP3A4 inhibitory effect of esomeprazole contributed to the net effect on *R*-pantoprazole metabolism when pantoprazole was given 1 hour after esomeprazole.

As the effects on midazolam and caffeine were evident in their metabolic ratios, our findings indicate that esomeprazole slightly inhibits CYP3A4 and induces CYP1A2, and that the effects were not driven by, for example, changes in gastric pH. Previously, 40 mg or 30 mg esomeprazole daily has had no significant effect on the pharmacokinetics of CYP3A4 substrates clarithromycin or quinidine, respectively, whereas 40 mg esomeprazole has increased the exposure to cisapride by 32%.[15] The current study using a sensitive CYP3A4 index substrate midazolam indicates that the hepatic CYP3A4 inhibitory effect of esomeprazole, even in the highest clinically used doses, is limited, probably only 25–30%, and declines to insignificant levels within 24 hours after dosing. Moreover, no signs of CYP3A4 induction were observed in any of the study phases. However, similarly to some studies with omeprazole,[13,15,33] a slight induction of CYP1A2 by high-dose esomeprazole was observed in

the present study. These effects on CYP3A4 and CYP1A2 activities can be clinically relevant in special situations, such as with anticancer agents or immunosuppressants with narrow therapeutic indices, or with drugs that are substrates for both CYP3A4 and 2C19.

In the current study, the CYP2C19 intermediate metabolizer phenotype (*CYP2C19*1/*2* genotype, 5/10 subjects) was over-represented, and CYP2C19 normal metabolizer phenotype (*CYP2C19*1/*1* genotype, 3/10 subjects) was under-represented, compared with the European population average.[2] Thus, it is possible that the mean effect of CYP2C19 inhibition on the pharmacokinetics of pantoprazole was smaller in the present study than it is in the general population with a lower frequency of no-function *CYP2C19* alleles. Interestingly, apart from a tendency to a stronger effect of esomeprazole on *R*-pantoprazole AUC in normal/rapid *CYP2C19* metabolizers (on average, > 5-fold increase in AUC) than in individuals carrying the *CYP2C19*2* allele, the estimated turnover half-life of CYP2C19 tended to be longer in normal/rapid *CYP2C19* metabolizers than in carriers of the *CYP2C19*2* allele (**Table S3**).

There are only few well-established *in vivo* CYP2C19 index substrates apart from omeprazole and *S*-mephenytoin. Omeprazole was naturally not suitable for this study, and, apart from its clinical hazards, *S*-mephenytoin is not commercially available in Europe. Pantoprazole, the index substrate used in the present study, is metabolized primarily by CYP2C19 and to a smaller extent by CYP3A4.[3,4] It has several advantages regarding pharmacokinetic studies, including good sensitivity and a short $t_{1/2}$, making it suitable for repeated testing of transient changes in enzyme activity. In previous studies, 80 mg dose of pantoprazole given simultaneously with clopidogel has decreased the AUC of clopidogrel's active metabolite, a sensitive index of CYP2C19 activity, only 14% suggesting negligible inhibitory effect on CYP2C19 activity at lower pantoprazole doses.[4] Furthermore, unlike omeprazole, pantoprazole displays similar pharmacokinetic characteristics after single and repeated dosing, indicating that it is not affected by increasing gastric pH[35,36] nor is it subject to autoinhibition of CYP enzymes. Even a ^{13}C-labeled pantoprazole breath test has been developed, providing a noninvasive method to assess CYP2C19.[37,38] The results of the current study reinforce the previous finding that *R*-pantoprazole is a sensitive marker for CYP2C19 activity.[17]

These findings indicate that when using the highest approved doses, esomeprazole can be classified as a moderate to strong CYP2C19 inhibitor, whereas extrapolations suggested weak to moderate inhibition at 20 and 40 mg twice daily dosing. Esomeprazole has a long-lasting inhibitory effect on CYP2C19 that can persist for at least 3–4 days following discontinuation of esomeprazole, indicating that irreversible inactivation is the main mechanism of this effect. Accordingly, even with the commonly used lower esomeprazole doses, care is warranted if a CYP2C19 substrate drug with narrow therapeutic index is used concomitantly or within a few days after discontinuation of esomeprazole. Additionally, high doses of esomeprazole cause a modest inhibition of CYP3A4 and induction of CYP1A2. Based on our estimate, the turnover half-life of CYP2C19 averages 53 hours. This estimate is useful for *in vitro-in vivo* extrapolations and physiologically-based pharmacokinetic modeling of drug-induced enzyme induction and mechanism-based inhibition of CYP2C19.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

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AUTHOR CONTRIBUTIONS

T.K., A.T., T.T., T.L., N.I., M.N., and J.B. wrote the manuscript. T.K., A.T., N.I., M.N., and J.B. designed the research. T.K., A.T., T.L., M.N., and J.B. performed the research. T.K., A.T., T.T., and J.B. analyzed the data.

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